GAS CHROMATOGRAPHY

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INTRODUCTION:-

- **Gas chromatography** – "It is a process of separating component(s) from the given crude drug by using a gaseous mobile phase."
- It involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Two major types:

- Gas-solid chromatography:

  Here, the mobile phase is a gas while the stationary phase is a solid.

  Used for separation of low molecular gases, e.g., air components, H_2S, CS_2 ,CO_2 ,rare gases, CO and oxides of nitrogen .

- Gas-liquid chromatography:

  The mobile phase is a gas while the stationary phase is a liquid retained on the surface as an inert solid by adsorption or chemical bonding.

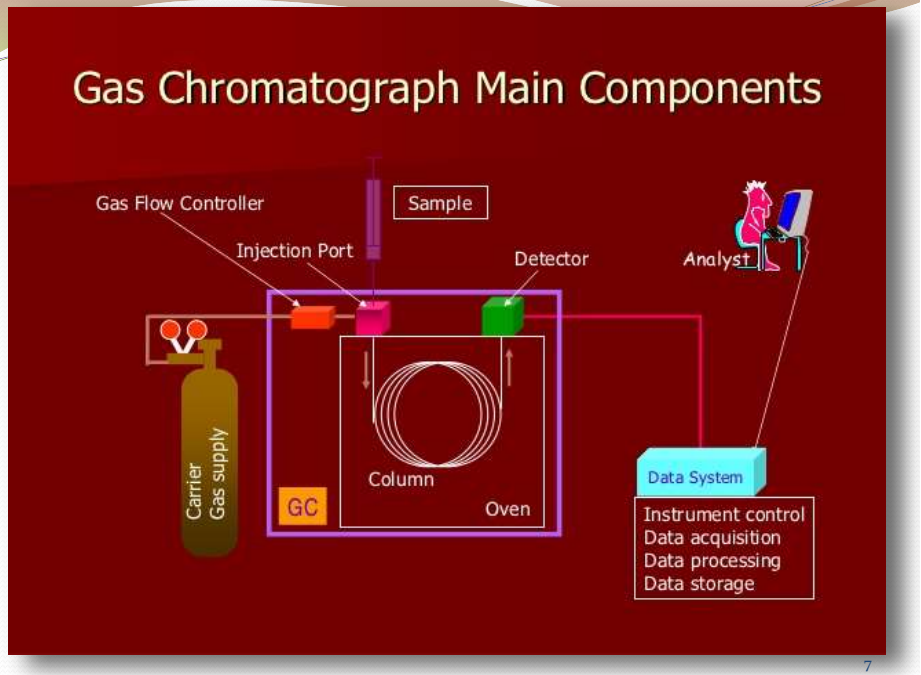
Principles:

- The principle of separation in GC is “partition.”
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.
- Partition coefficient is “the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.’

Instrumentation:-

- Carrier gas
  - He (common), N_2, H_2, Argon
- Sample injection port
  - micro syringe
- Columns
- Detectors

➢ Thermal conductivity (TCD)
➢ Electron capture detector(ECD)
➢ Flame Ionization detector (FID)
➢ Flame photometric (FPD)



Carrier gas:-

- The cylinder/ gas tank is fitted with a pressure controller to control the pressure of gas, a pressure gauge that indicates the pressure, a molecular sieve to transfer filtered dry gas and a flow regulator to ensure a constant rate of flow of mobile phase to the column.
- It should meet the following criteria:

✓Should be chemically inert
✓Should be cheap and readily available
✓Should be of high quality and not cause any fire accidents
✓Should give best possible results
✓Should be suitable for the sample to be analyzed and for the detector

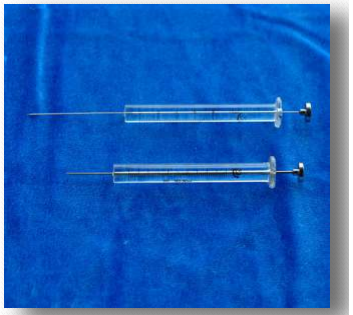
- Hydrogen, helium, nitrogen and carbon dioxide are commonly used.
- Hydrogen has low density and better thermal conductivity. However, it reacts with unsaturated compounds and is inflammable and explosive in nature.
- Nitrogen is inexpensive but it gives reduced sensitivity.
- He is the most preferred gas.
- Inlet pressure ranges from: 10-50 psi

  -Flow rate : 25-150 mL/min for packed columns

  -Flow rate: 2-25 mL/min for open tubular column

Sampling unit:-

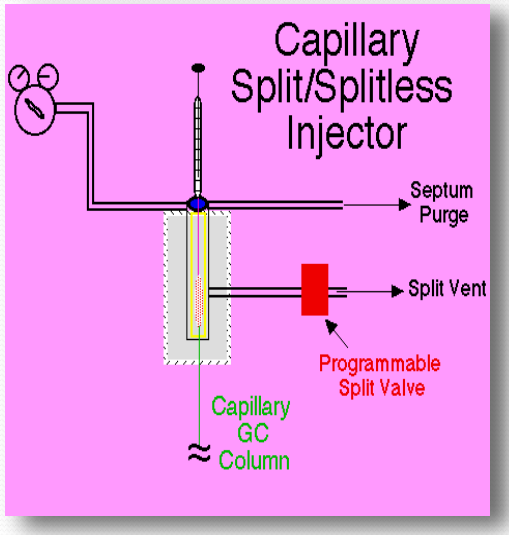
- Sampling unit or injection port is attached to the column head.
- Since the sample should be in vapourized state, the injection port is provided with an oven that helps to maintain its temperature at about 20-50° C above the boiling point of the sample.
- Gaseous samples may be introduced by use of a gas tight hypodermic needle of 0.5-10 ml capacity.
- For Liquid samples , micro syringes of 0.1-100µL capacity may be used.



Microsyringe

Injections of samples into capillary columns:-

a. Split injections- it splits the volume of sample stream into two unequal flows by means of a needle valve , and allow the smaller flow to pass on to the columns and the bigger part is allowed to be vented to the atmosphere. This technique is not suitable when highest sensitivity is required.



b. Splitless injectors- They allow all of the sample to pass through the column for loading. Sample should be very dilute to avoid overloading of the column and a high capacity column such as SCOT or heavily coated WCOT columns should be used.

c. On column injectors: A syringe with a very fine quartz needle is used. Air cooled to -20°c below the b.p. of the sample. After then the warmer air is circulated to vaporize the sample.

d. Automatic injectors: For improving the reproducibility and if a large number of samples are to be analyzed or operation is required without an attendant, automatic injectors are used.

- The solid samples are introduced as a solution or in a sealed glass ampoule, crushed in the gas stream with the help of a gas tight plunger, and the sample gets vapourized and flows into column under the influence of carrier gas.

Column unit:-

- Columns are of different shapes and sizes that includes: "U" tube type or coiled helix type.
- They are mainly made of copper, stainless steel, aluminium, Glass, nylon and other synthetic plastics.

➢ ***Support material*:-**

✓ it's main function is to provide mechanical support to the liquid phase. An ideal support should have a large surface area, chemically inert, should get uniformly wet with liquid phase, should be thermostable.

✓ Commonly used solid phases are: diatomaceous earth or kieselguhr, glass beads, porous polymers, sand,etc.

➢ *Liquid phase:-*

It should have the following requirements:

✓ It should be non-volatile

✓ Should have high decomposition temperature

✓ Should be chemically inert

✓ Should posses low vapour pressure at column temperature

✓ Should be chemically and structurally similar to that of the solute i.e., polar for polar solute.

✓ Examples of different liquid phases:

CATEGORY	EXAMPLES
Non-polar hydrocarbon phases	Paraffin oil (nujol), silicon oil, silicon rubber gum (used for high temp of about 400°
Polar compounds (having polar groups like -CN, -CO and –OH)	Polyglycols (carbowaxes)
Liquids having hydrogen bonding	Glycol, glycerol, hydroxy acids

➢Types of columns:-

- There are two general types of columns:

1. ***Packed columns***:- In GLC, they are densely packed with finely divided, inert, solid support material (*diatomaceous earth*) coated with liquid stationary phase.

   In GSC, the columns are packed with adsorbents or porous polymers.

✓ Length- 1.5 - 10m

✓ internal diameter- 2 - 4mm.

1. ***Capillary columns***-

✓ length ranges from 10-100m

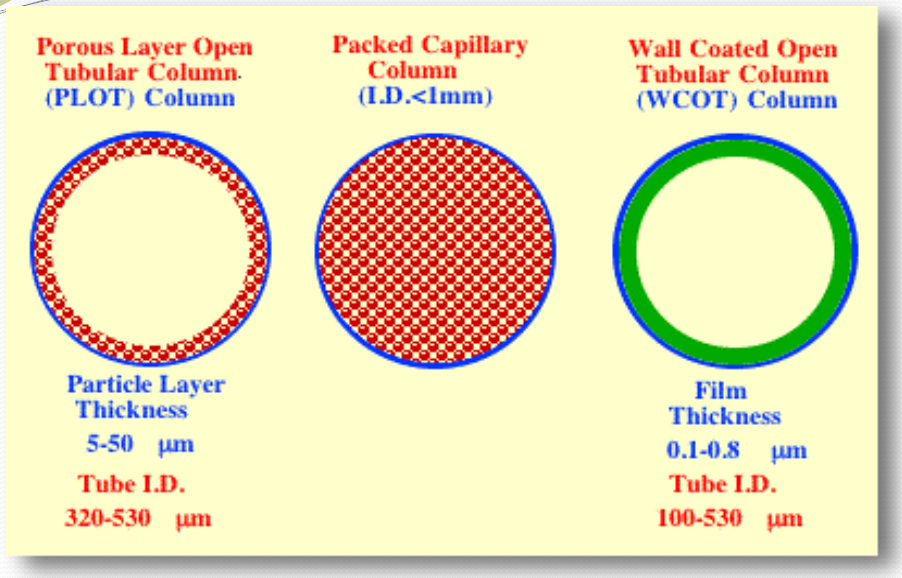
✓ inner diameter is usually 0.1-0.5mm.

- It is mainly of two types:

❑**Wall-coated columns -** consist of a capillary tube whose walls are coated with liquid stationary phase.

❑ **Support-coated columns-** the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. It is also known as PLOT (porous-layer open tubular columns).

➢SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.



➢*Equilibrium of the column:-*

- The packed columns are equilibrated before introduction of the sample. This is done by allowing continuous flow of heated carrier gas through the columns for a specific duration of time (24hrs) at prescribed temperature.
- Ideally prepared and conditioned columns show a zero base line on the recorder upon passage of carrier gas alone.

➢*Column temperature:-*

- This can be controlled by jackets equipped with vapours of a boiling liquid, electrically heated metal blocks or circulating air baths.
- Compounds of low B.P- eluted at lower temperature
- Compounds of high B.P- boils at higher temperature resulting in broader and shallower peaks, require temperature programming.

Detectors:-

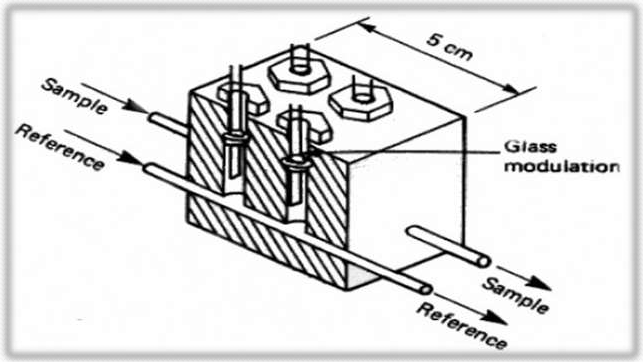
- The eluted solute particles along with the carrier gas exit from the column and enter the detector.
- The detector then produces electrical signals proportional to the concentration of the components of solute.
- The signals are amplified and recorded as peaks at intervals on the chromatograph.

Properties of an ideal detector:

- Sensitive
- Operate at high T (0-400 °C)
- Stable and reproducible
- Linear response
- Wide dynamic range
- Fast response
- Simple (reliable)
- Nondestructive
- Uniform response to all analytes

I. Thermal conductivity detector:-

➢ "TCD is based upon the fact that the heat lost from a filament depends upon the thermal conductivity of the stream of surrounding gas as well as its specific heat."



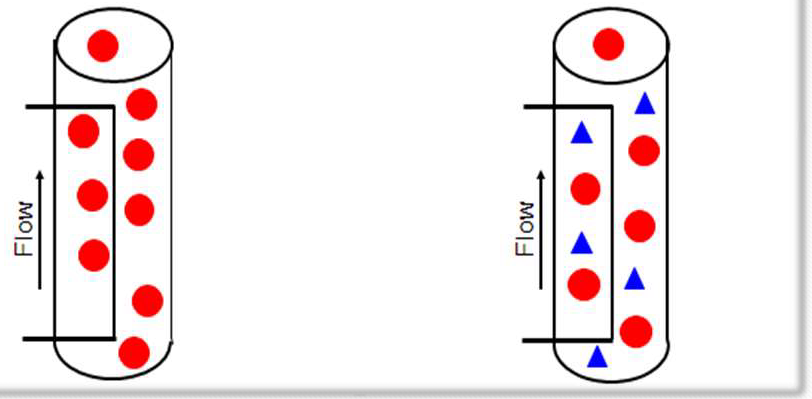
➢ When only carrier gas flows heat loss to metal block is constant, filament T remains constant.

➢ When an analyte species flows past the filament generally thermal conductivity changes, thus resistance changes which is sensed by Wheatstone bridge arrangement.

➢ The imbalance between control and sample filament temperature is measured and a signal is recorded.

The TCD is a nondestructive, concentration sensing detector. A heated filament is cooled by the flow of carrier gas.

When the carrier gas is contaminated by sample ▲, the cooling effect of the gas changes. The difference in cooling is used to generate the detector signal.



- Advantages :-
  - ➢ Simple and inexpensive
  - ➢ Durable and posses long life
  - ➢ Accurate results
  - ➢ Non-selective, hence known as universal detectors

- Disadvantages:-
  - ➢ Low sensitivity
  - ➢ Affected by fluctuations in temperature and flow rate.

II. Electron capture detector:-

- Molecules of compounds, which posses affinity for electrons, differ in their electron absorbing capacities. This difference is utilized in this detector for identification of the compounds.
- **Working**- A foil made up of a radioactive metal like Ni^{63} (β-emitter) is placed inside a Teflon coated cell which also contains a cathode and an anode.
- In the absence of organic species, the produced electrons migrate towards positive electrode and produce a certain constant standing current.
- When a sample/eluent is present it captures the electrons, elutes from column, there is a drop in this constant current.
- The potential across two electrodes is adjusted to collect all the ions and a steady saturation current, is therefore, recorded.

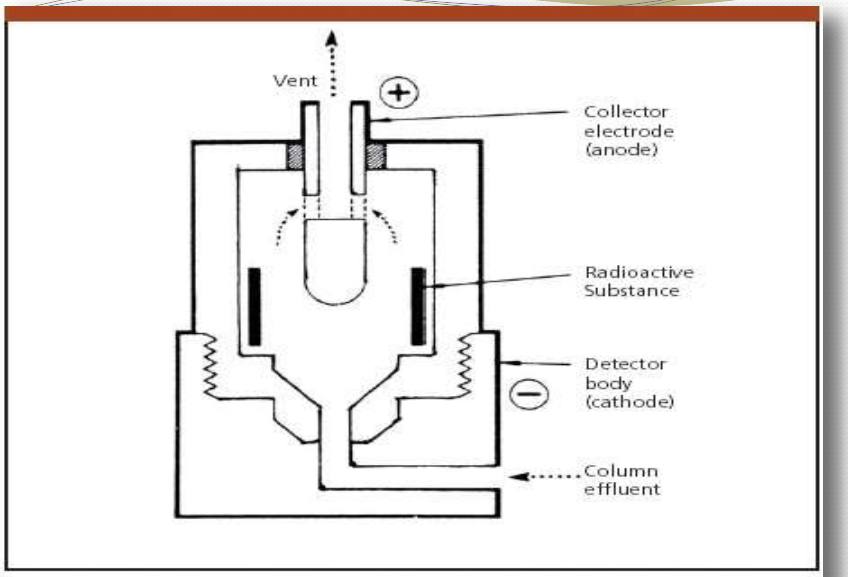


Figure 1: Schematic of a typical early electron-capture detector (2).

➢Advantages:-

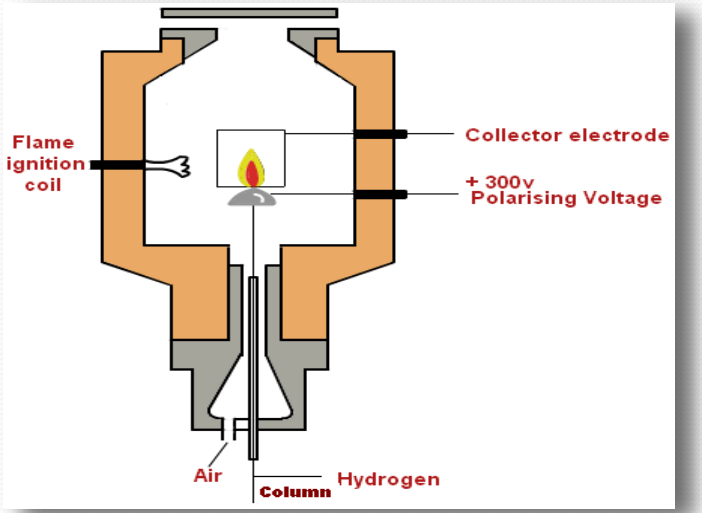
- Highly selective
- Highly sensitive for the detection of compounds like halogens, quinones, peroxides, nitrites, etc.
- It is non-destructive
- More sensitive than TCD and FID.

➢Disadvantages:-

- Least sensitive to compounds whose molecules have negligible affinity for electrons.
- Carrier gas used should be of pure form like pure nitrogen.

III.Flame ionization detector:-

- This employs hydrogen flame that is maintained in a small cylindrical jet made up of platinum or quartz.
- Effluent from the column with helium or nitrogen as carrier gas are fed into the hydrogen flame, gets ignited and undergoes pyrolysis to produce ions.
- For detection of these ions, two electrodes are used that provide a potential difference.
- The ions produced are repelled by the positive electrode which hit the collector plate. The current produced in doing so is amplified and fed to an appropriate recorder.

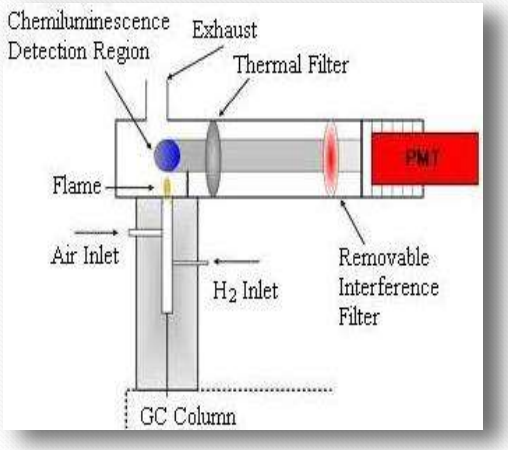


Flame ionization detector

IV.Flame photometric detector:-

- It is a selective detector that is responsive to compounds containing sulphur or phosphorous
- The detection principle is the formation of excited sulphur (S_2*) and excited hydrogen phosphorous oxide species (HPO*) in a reducing flame.
- A photomultiplier tube measures the characteristic chemiluminescent emission from these species. The optical filter can be changed to allow the photomultiplier to view light of 394 nm for sulphur measurement or 526 nm for phosphorus.

- Applications:
  -For detection of heavy metals like chromium, selenium, tin, etc, in organometallic compounds.
  -Also for analysis of pesticides, coal, hydrogenated products as well as air and water pollutants.



Flame photometric detector

Working:-

- Fill the syringe with sample.
- Record the setting i.e., column temperature, detector temperature and injection port temperature.
- Introduce sample into the injection port by completely inserting the needle into the rubber septum. Note down the injection time.
- The sample gets vapourized due to higher temperature of injection port and is swept into column by carrier gas.
- This sample components now get distributed between the gas and stationary liquid phase depending upon their solubilizing tendencies.
- The components with minimal solubility move faster and those with maximum solubility travel slowly.
- The components leaving the column activate detector and recorder to give a plot.

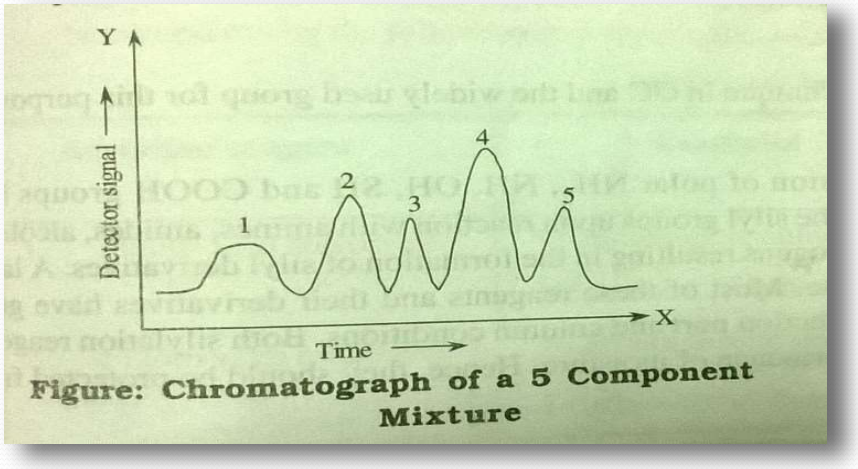


Figure: Chromatograph of a 5 Component Mixture

- The components that slowly leave the column give a bell shaped curve of shorter peak while the one which travels faster gives a bluntly pointed curve of larger peak.
- In above graph, the component that first emerges out of the column is component 4 followed by 2,5,3 and 1.
- The area under the curve is determined in order to obtain the percentage composition of the mixture.

Evaluation :-

- HETP (height equivalent to theoretical plate)- It is the distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in liquid phase. Larger the number of theoretical plates/ smaller the HETP, the more efficient the column is for separation.

  HETP = Length of column/n ; Where n = number of theoretical plates
- Retention Time: defined as the absolute time taken by a sample to show maximum peak after injecting.
- Retention Volume: defined as the volume of gas required to elute about half of the solute through the column.

  $V_R = t_R \times F$

  F =average volumetric flow rate (mL/min) ,estimated by using soap bubble meter (some gases dissolving in soap solution)

Applications:-

- *Qualitative Analysis* – by comparing the retention time or volume of the sample to the standard / by collecting the individual components as they emerge from the chromatograph and identifying these compounds by other methods like UV, IR, NMR.
- *Quantitative Analysis*- area under a single component elution peak is proportional to the quantity of the detected component/response factor of the detectors. It is done by:

I. Direct comparison method-

$$A_{(sample)} / A_{(std)} = \alpha\, C_{(sample)}/C_{(std)}$$

Where, α is the response factor determined for every pure compound under given conditions.

II. Calibration curve- a graph is plotted by taking peak areas on Y axis and concentration of standard compound on X axis. Concentration of unknown sample is then determined by plotting its peak area on same graph.

III. internal standard method- A known concentration of internal standard, which has similar retention characteristics as that of sample is added to both reference standard and test sample.

- *Pharmaceutical applications-*

➢ Quality control and analysis of drug products like antibiotics (penicillin), antivirals (amantidine), general anesthetics (chloroform, ether), sedatives/hypnotics (barbiturates), etc.

➢ Assay of drugs – purity of a compound can be determined for drugs like :

✓ Atropine sulphate

✓ Clove oil

✓ Stearic acid

➢ In determining the levels of metabolites in body fluids like plasma, serum, urine, etc.

- *Miscellaneous:*

➢ analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drug and pesticides residues, trace elements.

➢ Pollutants like formaldehyde, carbon monoxide, benzene, DDT etc.

➢ Dairy product analysis like milk, butter –for detection of aldehydes, milk sugars, ketones and fatty acids.

➢ Separation and identification of volatile materials, plastics, natural and synthetic polymers, paints, and microbiological samples.

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